

## COMBINATION OF SAWDUST, “FILTER CAKE” AND CALCIUM CARBONATE AS GROWTH MEDIUM FOR THE PRODUCTION OF WHITE OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*)

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### ABSTRACT

This study was aimed to investigate the effect of combining sawdust (SD), filter cake (FC) and calcium carbonate as growth medium for the production of white oyster mushroom. Isolate F2 oyster mushroom was cultured on malt extract agar (MEA) and used in the experiment. The culture medium consisted of two treatments: first, A treatment: combined medium ( $A_0=100\%$  SD;  $A_1=100\%$  FC;  $A_2=70\%$  FC and  $30\%$  SD;  $A_3=50\%$  FC and  $50\%$  SD;  $A_4=30\%$  FC and  $70\%$  SD) and second, K treatment: addition of calcium carbonate ( $K_1=2\%$ ;  $K_2=3\%$ ;  $K_3=4\%$ ;  $K_4=5\%$  weight of medium). A Randomized design was used to analyze certain parameters, such as mycelial growth, presence of fruiting body, the number of fruiting bodies and the fresh weight of fruiting body at harvest. The results showed that the highest mycelial growth and fruiting body formation occurred on  $A_0$  treatment. However, a high number of fruiting bodies and a high fresh weight of fruiting body at harvest were obtained on  $A_4$  treatment. Interaction between the combined medium (A) and addition of calcium carbonate (K) showed that the highest mycelial growth occurred on  $A_0K_2$  treatment 30 days after incubation. The composition of  $A_4$  treatment (30% FC and 70% SD) was found to be the optimal medium for the production of fruiting body. This finding shows that FC with additional nutrition could as a substitute of SD medium for cultivating white oyster mushroom.

**Keywords:** growth medium; mycelial growth; white oyster mushroom; fruiting bodies

مارديانا وآخرون

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مزيج من نشارة الخشب وكربونات الكالسيوم كوسط نمو انتاج فطر المحار الأبيض

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المستخلص

هدفت الدراسة إلى معرفة تأثير الجمع بين نشارة الخشب (SD) وكعكة الترشيح (FC) وكربونات الكالسيوم كوسيط نمو وانتاج فطر المحار الأبيض. استزرع فطر المحار المعزول F2 على سيقان مستخلص الشعير (MEA) واستخدم في التجربة. يتكون وسط الاستزراع من معاملتين : أولاً ، وسط مشترك ( $A_0 = 100\%$  SD ؛  $A_1 = 100\%$  FC ؛  $A_2 = 70\%$  FC و  $30\%$  SD ؛  $A_3 = 50\%$  FC و  $50\%$  SD ؛  $A_4 = 30\%$  FC و  $70\%$  SD) والثاني معاملة K: إضافة كربونات الكالسيوم ( $K_1 = 2\%$  ؛  $K_2 = 3\%$  ؛  $K_3 = 4\%$  ؛  $K_4 = 5\%$  وزن متوسط). تم استخدام التصميم العشوائي لتحليل ، مثل النمو الفطري ، ووجود الجسم الثمر ، وعدد الأجسام الثمرية والوزن الطازج لجسم الثمار عند الحصاد. أظهرت النتائج أن أعلى نمو فطري وتكوين جسم ثمر حدث في علاج  $A_0$ . ومع ذلك ، تم الحصول على عدد كبير من أجسام الثمار ووزن طازج مرتفع لجسم الثمار عند الحصاد على معاملة  $A_4$ . أظهر التفاعل بين الوسط المركب (A) وإضافة كربونات الكالسيوم (K) أن أعلى نمو فطري حدث في علاج  $A_0K_2$  بعد 30 يوماً من الحضانه. تم العثور على تركيبة المعاملة (30)  $A_4$  (FC و  $70\%$  SD) لتكون الوسيلة المثلى لإنتاج الجسم الثمرى. تظهر هذه النتيجة أن FC مع التغذية الإضافية يمكن أن تكون بديلاً لوسط SD لزراعة فطر المحار الأبيض.

الكلمات المفتاحية : متوسط النمو ؛ نمو فطري فطر المحار الأبيض اثمار الاجسام

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## INTRODUCTION

Mushroom is a horticultural commodity and is used as food or nutraceutical to prevent and treat human diseases. Preclinical and clinical studies have shown that direct and indirect consumption of certain mushrooms reduce the occurrence of cancer and cause weight loss (8). Indonesia is a country that has the potential to become an edible mushroom producer. As a tropical country, Indonesia has high mushroom diversity, which makes it possible to grow them in every season. Edible mushrooms with high nutritional value can be used as medicine (19). The high demand for mushroom as a food additive in the society and the increasing mushroom production indicate business opportunities in mushroom cultivation. Setyawati (23) stated that the presence of mushroom farming in some areas increases the value of the commodity. As the tenth largest producer of sugar-cane in the world, Indonesia produces a large amount of waste product in this sector. According to Kuswuri (14), the biomass of sugar-cane waste was 1.1 million ton/hectar/year. Currently, the waste is used primarily as fertilizer, alternative fuel source or abandoned, which causes pollution (9). The composition of filter cake was investigated by Senthil and Das (22), Kumar *et al.* (13), and Sørensen *et al.* (12). Boonyuen *et al.* (3) reported that filter cake and sugar-cane stem waste consist of high lignocellulose and can be used for fungal growth. A previous research by Suka *et al.* (6) stated that the addition of various amounts of filter cake to sawdust medium increases the production of *Pleurotus ostreatus*. They also reported impressive results with respect to mycelial growth, pinhead, fruiting body and harvest period for the medium with 60 g of filter cake. Addition of calcium carbonate ( $\text{CaCO}_3$ ) to filter cake is required to increase pH. Mardiana *et al.* (15) reported a difference in mycelial growth of *Pleurotus ostreatus* on a medium with  $\text{CaCO}_3$ . Therefore, the aim of the current research is to investigate the use of the combined medium of saw dust, filter cake and  $\text{CaCO}_3$  as growth medium for the production of white oyster mushroom (*Pleurotus ostreatus*).

## MATERIALS AND METHODS

Isolate F<sub>2</sub> white oyster mushroom was cultured on malt extract agar (MEA) obtained from F<sub>1</sub> Agrotechnology laboratory, Agricultural Faculty, Medan Area University and was used in this experiment. The treatments consist of A treatment, which is a combined medium of filter cake and sawdust (A<sub>0</sub>=100% SD; A<sub>1</sub>=100% FC; A<sub>2</sub>=70% FC and 30% SD; A<sub>3</sub>=50% FC and 50% SD; A<sub>4</sub>=30 FC and 70% SD), and K treatment: addition of calcium carbonate include K<sub>1</sub>=2%; K<sub>2</sub>=3%; K<sub>3</sub>=4%; K<sub>4</sub>=5% weight of medium, which involves the addition of  $\text{CaCO}_3$ . A Randomized design was used to analyze certain parameters, such as mycelial growth, the presence of fruiting body, the number of fruiting bodies and the fresh weight of fruiting body at harvest. The experiment using randomized complete design, with three replication analysis of variance (ANOVA) and Duncan multiple range test (DMRT) was formulated as follow:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \tilde{E}_{ijk}$$

### Medium Preparation, Isolation and Incubation

Isolate F<sub>2</sub> oyster mushroom was obtained from F<sub>1</sub> culture collection Agrotechnology Laboratory, Agriculture Faculty, Medan Area University. The isolate was cultured on baglog with a volume of 375 cm<sup>3</sup> containing powdered filter cake, sawdust, wheat bran, 10 %  $\text{CaCO}_3$  and 0.5% maize flour. Eighty baglogs were made in this experiment. Each baglog was arranged and incubated (25-28°C) for 35-40 days in the dark on a rack in a mushroom house, 7 x5 x 6 m (length, wide, height). Each treatment was made in triplicate. Inoculated eight baglogs with spawn were made for each treatment. The humidity inside the mushroom house (80-90 %) was controlled daily using a hygrometer. To control pest and diseases in each treatment, fly trap was used.

### Parameter Observation

Each baglog was observed after 3 days of incubation. Certain parameters, such as mycelial growth, were observed after 5 days of incubation. The size of the fruiting body was observed after 35-40 days, while the number of fruiting bodies and the fresh weight were observed at harvest. Harvesting was conducted two times between 40 and 120 days of incubation using cutter (11). The first harvest

took place on the 50th day and the second on the 75th day. The freshly harvested mushroom was weighed using analytical balance type Gs/Vibra, Merk Shinko, Japan.

## RESULTS AND DISCUSSION

The medium composition of each baglog was covered by mycelial growth after 35 days. The combined medium with  $\text{CaCO}_3$  supports fungal growth. Previous results showed that sawdust and filter cake contain cellulose, which is required by white oyster mushroom for growth (2). Garg and Gupta (9) stated that agro industrial wastes and their derivatives were used as medium for cultivating oyster mushroom. The high lignocellulose produced by mushroom gives the species advantage in growth and development on such environment. In addition to light intensity, the temperature, acidity/alkalinity of the medium (6-7) and relative humidity (60-70%) in the mushroom house affect their growth. Soenanto (18) reported that oyster mushrooms require an area that is 400-800 m above sea level for optimum growth. However, some strains of the species are able to grow on lower than 400 m above sea level as long as the area has low light intensity and cool weather. Our results are in accordance with Chazali and Pratiwi (4), who stated that a temperature range of 23-28°C was optimal for mycelial growth of oyster mushroom. High light intensity inhibits growth and metabolism. Suriawiria (2) stated that fungal growth was affected by the nutrients on the medium, particularly phosphorus, potassium, nitrogen, sulphur, calcium and carbon. Previous research by Soenanto (18) and Hanifah (10) showed that the nutrient and moisture contents of the medium determine fungal growth. The result of this study was showed that the presence of nutrients on sawdust and filter cake, the moisture content of both media (60-65%) and their pH (6-7) determine the growth of oyster mushroom.

## Mycelial growth covered the Substrat/baglog (cm)

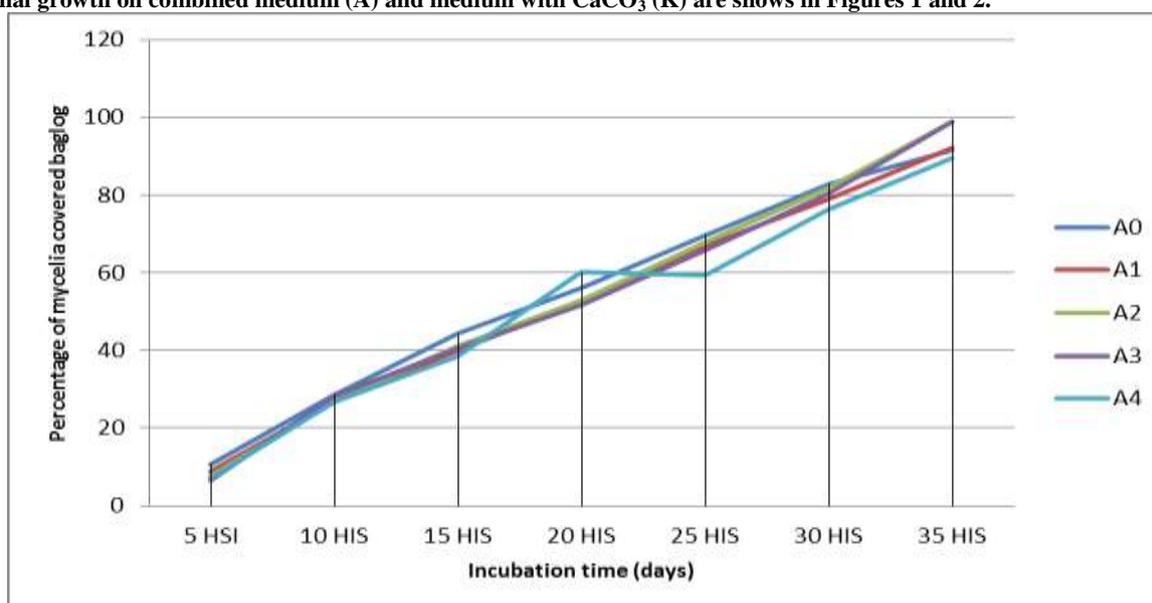
The statistical analysis indicate that the combined medium (sawdust, filter cake and  $\text{CaCO}_3$ ) had a significant effect ( $P < 0.05$ ) on mycelial growth. We assumed that the starting point of mycelial formation was determined by the presence of nutrients in the medium in the baglog. Optimum mycelial growth occurred after 30 days.  $A_0$ ,  $A_2$ , and  $A_3$  had no significant effect on mycelial growth. The result indicates that filter cake can be used as a substitute medium of sawdust. The optimum growth of mycelium with respect to the medium containing  $\text{CaCO}_3$  was observed on  $K_2$ ; the performance of  $K_2$  is significantly different from those of others. Interaction  $A_0K_2$  showed optimum mycelial growth. Sawdust and filter cake contain nutrients required for optimum growth of white oyster mushroom. Similar results were also reported by Dewi (5). Agus *et al.* (1), Fadjar (7) and Mkhize *et al.* (17) reported that sawdust consists of 33-38% of cellulose, 15-25% of hemicellulose, and 18-33% of lignin. However, filter cake consist of carbon (26.51%), nitrogen (1.04 %), C/N (25.62), phosphate (6.142%), potassium (0.485 %), sodium (0.082%) calcium (5.785%), magnesium (0.419%), iron (0.191%) and mangan (0.115%). Nitrogen as well as carbohydrate and its derivatives are essential compounds for mycelial growth and fruiting body formation of oyster mushroom. Winarni and Rahayu (20), reported that digested carbohydrate by fungal enzymes provide nutrients in the culture medium. Mkhize *et al.* (17) observed that mycelial growth and short period mycelial colonization of *Pleurotus ostreatus* occurred with high concentration of maize flour and wheat bran. Due to its growth, the mycelia of oyster mushroom cover all the substrates in the baglog. The process occurred 5 to 35 days after inoculation (Table 1).

**Table 1. Mycelial growth of oyster mushroom on *baglog* containing filter cake, sawdust and calcium carbonate after 5-35 days incubation (29°C) in a mushroom house**

Treatme nt	Length of mycelia (cm) on baglog/days of incubation						
	5	10	15	20	25	30	35
A0	3.19aA	8.56aA	13.26Aa	16.81tn	20.91aA	24.84aA	27.5Cc
A1	2.68bAB	8.03cA	11.95cCD	15.85tn	20.1bcAB	23.7cdB	27.66cC
A2	2.3 bcB	8.09bcA	12.34abB	15.82tn	20.38abA	24.56bA	29.69 aA
A3	1.92cC	8.42abA	12.11bcBC	15.53tn	19.68cdB	24.1bcAB	29.63abAB
A4	2.12cC	7.97cA	11.52cD	18.05tn	17.8dB	22.89dB	26.88Cc
K1	2.59tn	8.28tn	12.24bA	15.8tn	19.58bA	23.95Bb	28.28tn
K2	2.52tn	8.36tn	12.46aA	15.98tn	20.44aA	24.8Aa	28.15tn
K3	2.29tn	8.13tn	12.13cA	15.58tn	19.32bA	23.61cC	27.98tn
K4	2.38tn	8.09tn	12.12cA	18.28tn	19.76bA	23.75 bcBC	28.68tn
A0K1	3.31aA	8.7abA	13.05bB	16.75tn	20.34cB	24.25bB	28.13tn
A0K2	2.99aA	9.59aA	14.63aA	18.15tn	22.94aA	27.63aA	26tn
A0K3	2.61bA	7.5cB	12.15dC	15.53tn	18.06dBC	22.38cC	25.88tn
A0K4	3.85aA	8.46bAB	13.21bB	16.8tn	22.31abA	25.13bB	30tn
A1K1	2.66bA	7.7cB	11.75eD	15.8tn	19.13cdB	22.88deC	27.13tn
A1K2	2.84bA	8.04bB	11.88eCD	16.26tn	20.31cB	24.19bB	27.75tn
A1K3	2.33cA	7.83bcB	11.99dC	15.4tn	19.94cB	23.56cC	27.63tn
A1K4	2.9abA	8.55bA	12.18dC	15.94tn	21.03bA	24.19bcB	28.13tn
A2K1	2.24cA	8bB	12.13dC	14.99tn	19.56cB	24.56bB	29.38tn
A2K2	3.03aA	7.94bB	12.56cB	16tn	21.13bA	24.56bB	30tn
A2K3	2.46bcA	8.56bA	12.53cdBC	15.98tn	20.44cA	24.63bB	29.38tn
A2K4	1.48eA	7.88bB	12.16dC	16.3tn	20.38cAB	24.5Bb	30tn
A3K1	2.16cA	8.63bA	12.76bcB	16.46tn	20.75bA	25Bb	30tn
A3K2	1.29eA	8.38bB	12.25dC	15.56tn	20.5bcA	25Bb	30tn
A3K3	2.08dA	8.56bA	11.9deC	15.5tn	19.2cB	23.94cBC	29.13tn
A3K4	2.15cdA	8.13bB	11.54eD	14.61tn	18.25dB	22.63cC	29.38tn
A4K1	2.55bA	8.38bB	11.49efD	15tn	18.13dB	23.06dC	26.75tn
A4K2	2.48bA	7.88bB	11fD	13.94tn	17.31deC	22.63cC	27tn
A4K3	1.96deA	8.19bB	12.06dC	15.5tn	18.94dB	23.56cdC	27.88tn
A4K4	1.5eA	7.4cB	11.53eD	27.75tn	16.81eC	22.31eC	25.88tn

Numbers followed by the same letters in a column are not significantly different at 0.95 (small letters) and 0.99 (capital letters)

Mycelial growth on combined medium (A) and medium with CaCO<sub>3</sub> (K) are shows in Figures 1 and 2.



**Figure 1. Mycelial growth covered baglog containing combined medium 5 – 35 days after incubation (29°C)**

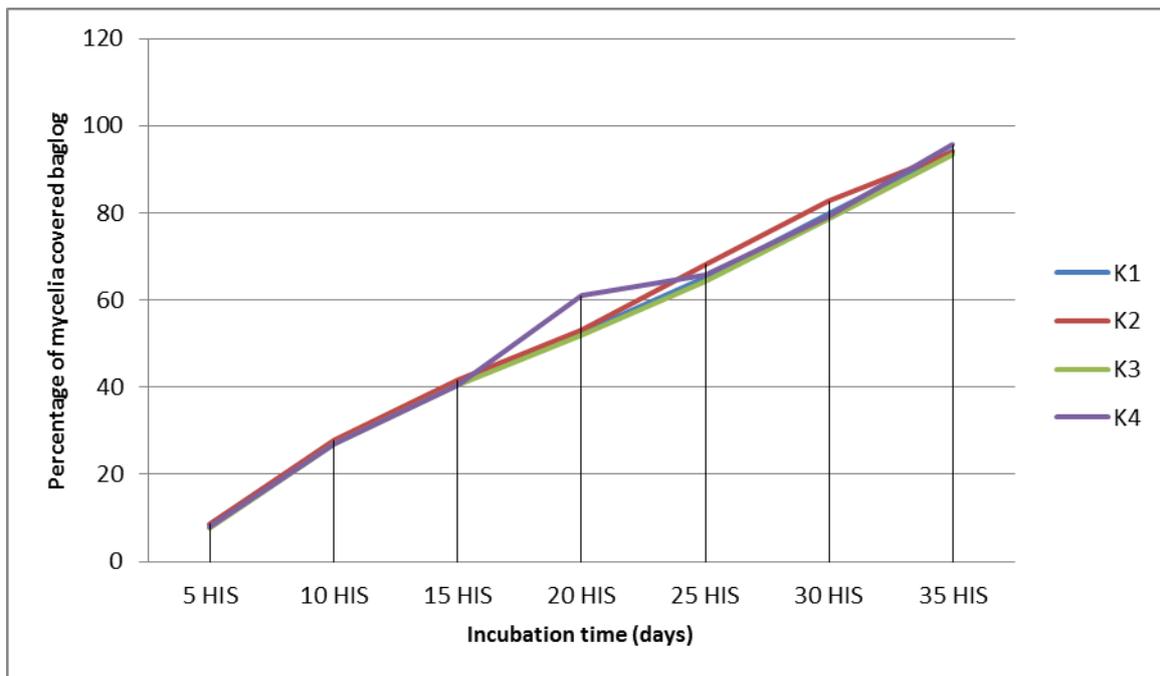


Figure 2. Mycelia growth covered baglog containing calcium carbonate 5 – 35 days after incubation (29°C)

The percentage of mycelial growth on baglog containing substrates and calcium carbonate as shows in Figure 3

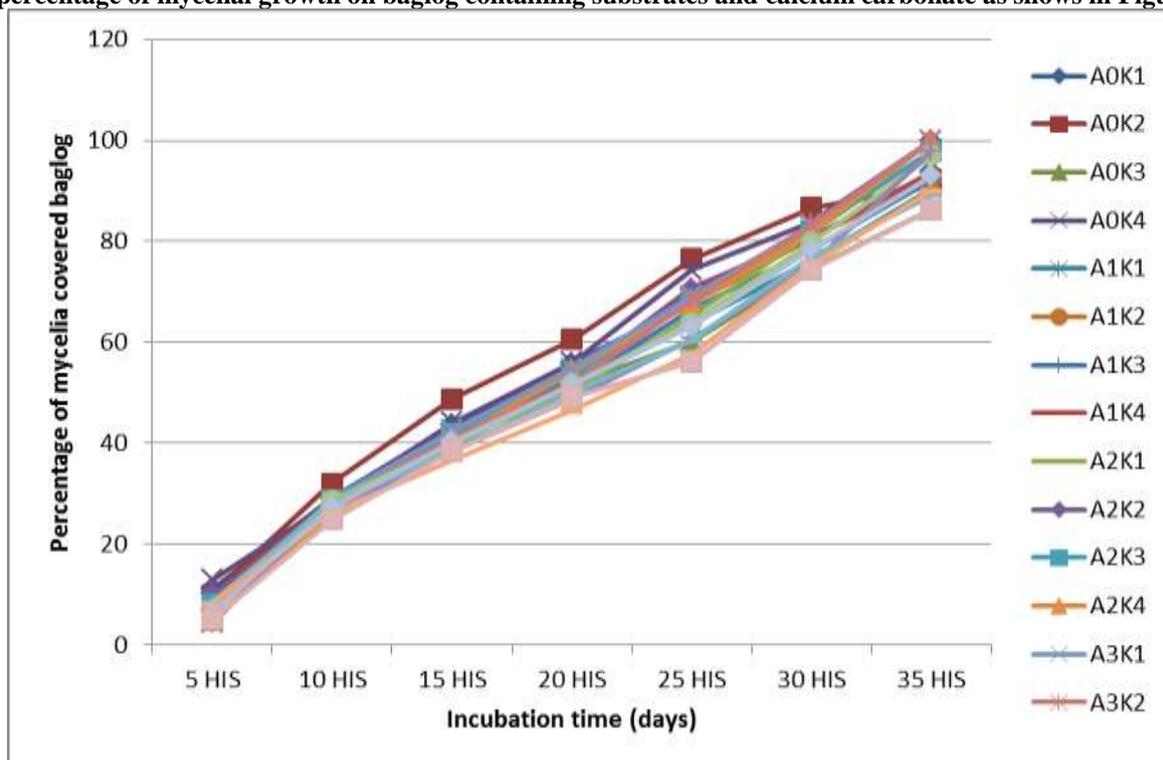


Figure 3. Percentage of mycelial growth on baglog containing substrates and calcium carbonate after 5 – 35 days incubation

**Formation of Fruiting Body**

The formation of fruiting body at the first and second harvests on 100% sawdust (SD) medium (A<sub>0</sub>) did not significantly different compared with the combined medium of 50% SD and 50% FC (A<sub>3</sub>). The table shows yield increase in the first harvest significantly with A<sub>4</sub> 60.88 aA and second harvest significantly

with A<sub>1</sub> and A<sub>2</sub>. The beginning of fruiting body formation is indicated by the presence of small white buttons surrounding the mycelia. The first appearance of fruiting body during the first and second harvests is shown in Table 2. The availability of nutrients, such as C, N, P, and K, on FC + SD medium promotes the growth of mycelia to the extent of covering the

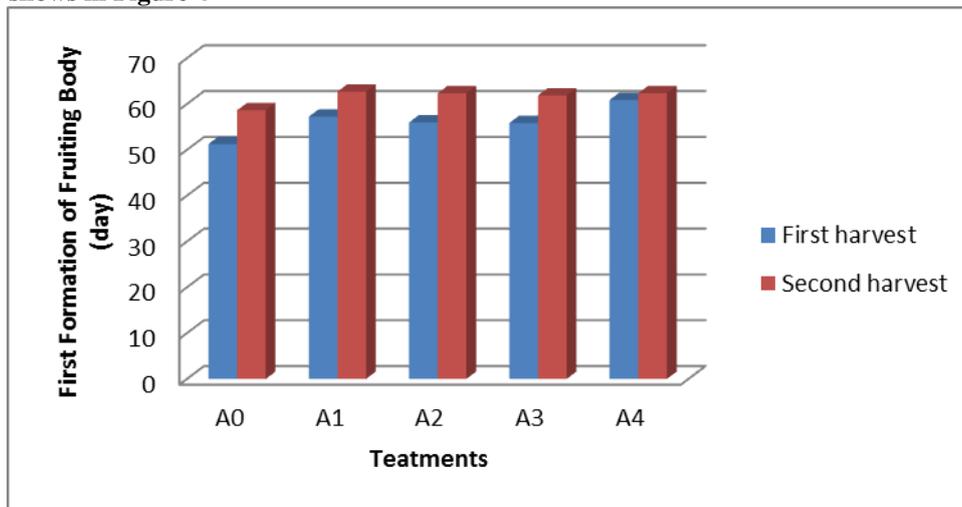
medium and enhances fruiting body formation. Maize flour and wheat bran added to the medium has no effect on fungal production.

**Table 2. The production of fruiting body of oyster mushroom on sawdust, filter cake and calcium carbonate at first and second harvests**

Treatment	The production of fruiting body (g)	
	First harvest	Second harvest
A <sub>0</sub>	51.28 cC	58.63 bB
A <sub>1</sub>	57.22 bAB	62.69 aA
A <sub>2</sub>	55.97 bB	62.34 Aa
A <sub>3</sub>	55.84 bcBC	61.88 abAB
A <sub>4</sub>	60.88 aA	62.34 abAB

Numbers followed by the same letters in a column have no significant difference at p<0.05 (small letters) and 0.01 (capital letters)

The first formation of fruiting body in all medium compositions occurred between 51.28-60.88 days and 58.63-62.69 days, as shows in Figure 4



**Figure 4. The first formation of fruiting body on some treatments of culture medium**

**Number of Fruiting Bodies**

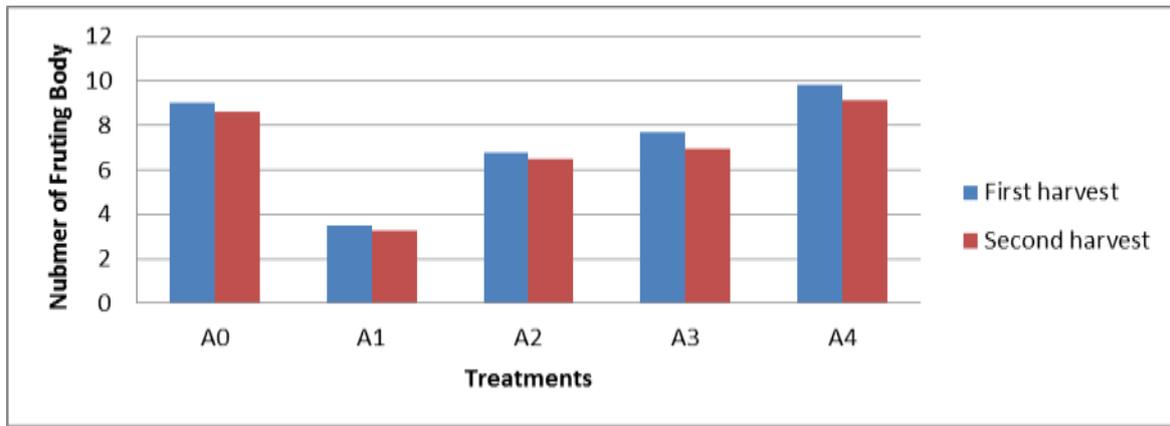
The number of fruiting bodies at the first harvest was 3.5-9.81 and that of the second harvest was 3.25-9.16 (Table 3 and Figure 5). The highest number of fruiting bodies was found on combined medium of 30% FC + 70% SD (A<sub>4</sub>), and did not significantly different from the number of fruiting bodies on the 100% SD medium (A<sub>0</sub>), 70% FC + 30% SD medium (A<sub>2</sub>) and 50% FC + 50% SD medium (A<sub>3</sub>). Regarding the production of fruiting body, filter cake was used as a substitute of

sawdust. The nutrients and compounds required for growing oyster mushroom include calcium, potassium, phosphorus, nitrogen, carbon, protein and chitin. Mkhize *et al.* (17) used nitrogen and carbohydrate for the production of fruiting body. However, Suriawiria (2) and Hanifah (10) reported that wheat bran and filter cake enriched the medium in terms of nutrients required for the growth of oyster mushroom, and the addition of calcium carbonate controlled the pH of the medium.

**Table 3. Number of Fruiting body of oyster mushroom at the first and second harvests of the combined medium of sawdust, and filter cake**

Treatment	Production of fruiting body	
	First harvest	Second harvest
A <sub>0</sub>	9 abAB	8.63 abcABC
A <sub>1</sub>	3.5 dc	3.25 d D
A <sub>2</sub>	6.75 cdC	6.47 abcdABC
A <sub>3</sub>	7.69 abcABC	6.97 abAB
A <sub>4</sub>	9.81 a A	9.16 aA

Numbers followed by the same letters in a column have no significant difference at 95% (small letter) and 99% (capital letter)



**Figure 5. Production of fruiting body at the first and second harvests**

**Fresh Harvest Weight**

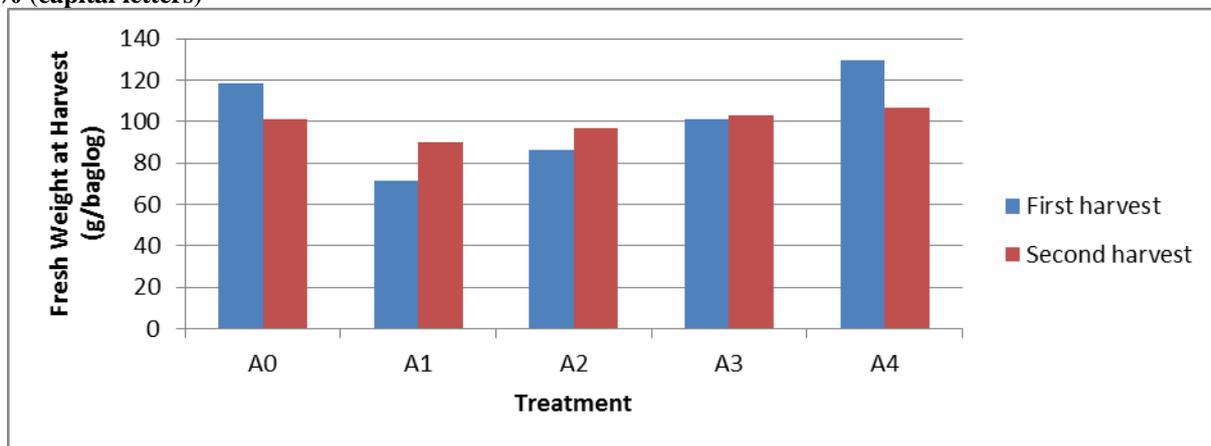
The fresh weight of fruiting body (g/baglog) at harvest is show in Table 4 and Figure 6. High harvest fresh weight was observed on A<sub>4</sub> in first and second harvest. With higher concentration of filter cake in the culture medium, the fresh weight increases. Filter cake

consists of crude protein, sugar, cellulose, chlorine, phosphate and fibre (5). Silveira *et al.* (21) reported that the presence of cellulose, hemicellulose and lignin in culture medium promotes mycelial growth, which increases fruiting body formation.

**Table 4. Fresh weight of fruiting body of oyster mushroom at first and second harvests regarding the medium containing sawdust, and filter cake**

Treatment	Fresh weight of fruiting body (g/baglog)	
	First harvest	Second harvest
A <sub>0</sub>	118.39 abAB	100.95 abcABC
A <sub>1</sub>	71.72 d D	90.16 d C
A <sub>2</sub>	86.5 cdCD	96.72 bcdABC
A <sub>3</sub>	101.2 bcBC	102.83 abAB
A <sub>4</sub>	129.83 Aa	106.75 a A

Numbers followed by the same letters in the same column have significant difference at 95% (small letter) and 99% (capital letters)



**Figure 6. Fresh weight of fruiting body at first and second harvests**

The results showed that the combined medium of sawdust and filter cake increases the productivity and growth of white oyster mushroom. The nutrients in sawdust were complemented by the addition of filter cake to promote fungal growth (16). As a sapotroph, the nutrients required by fungi are provided by other organisms. Environmental factors such as light intensity, temperature, acidity/

alkalinity of medium and humidity also affect fungal growth.

**Conclusion**

As waste agricultural products, filter cake and sawdust used as a combined medium is suitable for cultivating white oyster mushroom. The combination of both waste products has a beneficial effect on mycelial growth and fruiting body formation.

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